Oxygen starvation promotes pro-tumorigenic cytokine expression

Identification of the factors that trigger tumor formation and metastasis is a problem that has persisted in cancer research since its inception. To date, studies have focused on chemical signaling compounds that are part of the secretome of cells. These signaling compounds trigger changes in cell proliferation and density, as well as function in the regulation of phenotypic expression, including cell movement and migration. These behavioral variations occur in response to activation of multiple signaling pathways in response to interleukin 6 (IL-6) and interleukin 8 (IL-8) expression. The phenotypic changes that occur in response to IL-6/IL-8 mediated pathway activations result in altered cell morphology and behavior. These changes include the formation by monolayer cells with dendritic projections accompanied by an increase in cell migration. A mostly overlooked aspect of the cell signaling system and the resultant metabolic response is the impact of gaseous signaling molecules, e.g. oxygen, on metastasis and tumor formation.

Recently, there has been a paradigm shift in the role of oxygen in disease and normal metabolism. It is now recognized that the normal levels of oxygen in the body range from 0% to around 12%; thus, the internal status of humans, or other animals, is hypoxic to anoxic. To date, the vast majority of cancer research ignores bio-relevant oxygen levels and continues to be carried out in carbon dioxide-enriched (5%) air containing 21% atmospheric oxygen levels. More recently, there has been an effort to examine cell metabolism in the presence of below atmospheric oxygen levels (hypoxia), or in 3D culture conditions which begin to mimic tumor formation. However, what these studies neglect to recognize is that the interiors of solid tumors are largely anoxic, due to a combination of high cell density and lack of vascularization.

In our previous studies characterizing anaerobic cell morphologic changes during long-term growth (17 days) in the absence of oxygen, we observed that anoxic cell morphology (dendritic projection appearance) resembled that reported for high cell density 3D triple negative metastatic breast cancer cells (MDA-MB-231). Such high density 3D cultures would exhibit oxygen gradients. Unfortunately, the level of oxygen deprivation required to alter expression of IL-6/IL-8 cannot be discerned from 3D cultures. Our study measured the direct contribution of oxygen deprivation to cytokine production. We determined that metabolically active anaerobic HeLa cells respond to the lack of oxygen by regulating cytokine expression. Oxygen starvation (anaerobic culture) significantly promoted expression of IL-6, IL-8, GRO, and IL-11 by hypoxia inducible factor, *HIF1* positive (3 days of anoxic culture) and/or *HIF1* negative (10 days anoxic culture; anaerobic respiration) cells, as compared to normoxic controls (5% CO_2 in air). In contrast, G-CSF, IFN?2, and CXCL-10 levels were depressed over time. These findings directly show that oxygen starvation corresponds to a secretome profile associated with metastasis. Further studies defining physiologic changes that occur upon anoxic growth may lead to the discovery of novel chemotherapeutic agents.

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Publication

Differential expression of cytokines and receptor expression during anoxic growth.
Plotkin BJ, Sigar IM, Swartzendruber JA, Kaminski A, Davis J
BMC Res Notes. 2018 Jun 25